STUDIES ON SEED PATHOLOGY OF CERTAIN VEGETABLES

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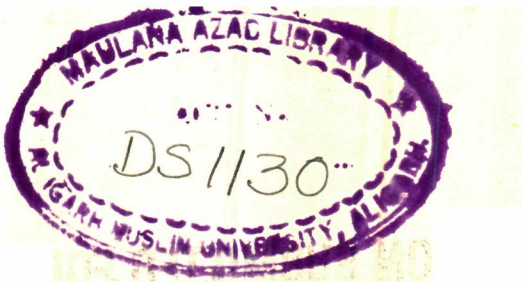
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I N T R O D U C T I O N

INTRODUCTION

Vegetables are natural sources of proteins, carbohydrates, vitamins and minerals, therefore, occupy an important position in the human diet, particularly in India where major population is vegetarian. Diseases and pests are responsible for heavy damages to vegetable every year (Walker,1952 and Prem Nath,1976). Amongst the various diseases, seed-borne diseases are of no less importance. Considerable work has been done on the seed pathology of vegetable seeds (Neergard,1977). But in all these studies one thing emerges that amongst various types of pathogens reported from seed, fungi constitute a major portion causing seed rots, seedling rot i.e. pre-and post-emergence damping off losses at various stages of plant growth.

Infection of vegetable to the extent of 50 percent has been reported in many areas of the world. Cramer (1967) pointed out that although statistical data on world wide losses are not available for the majority of vegetables, but it is felt that losses are enormous. In the U.S.A. annual average loss of bean (Phaseolus vulgaris) was 20 percent of which 16 to 17 percent was due to seed borne infection (Anon,1965) and of these one third was attributed to Fusarium solani f. sp. phaseoli alone. In 1939 Fusarium

root rot caused 95 percent losses of beans in New South Wales, Australia (Anon,1941). In South Western Colarado 84 percent yield reduction was recorded to vegetable crops and not a single field sample was found free of the disease (Keenan et al.,1974). In temperate countries Collectotrichum lindemuthianum the causal agent of bean anthracnose, a seed-borne fungus, has been reported to cause tremendous loss to beans. Masurat et al.(1966) reported that the damage of beans to the extent of five percent was due to anthracnose during 1965 in East Germany. While Chang (1960) observed 50 - 54 in U.S.S.R. Losses upto 50 percent have been reported in the U.S.A. (Zaumeyer and Thomas,1957) and in recent years losses have increased alarmingly. In Western Nebraska the average loss in dry beans was 6 to 19 percent in different years from 1970 - 72 (Kerr and Steadman,1974) while in New York it was 10 to 20 percent (Lumsden et al., 1974). Leaf spot causing pathogen Ascochyta spp. of beans and kidney beans was also found seed-borne and was reported causing considerable losses (Ondrej,1971). Watanabe (1972) isolated virulent Macrophomina phaseoli from commercial kidney bean seed lots. Where as, seed rot and root rot fungi Fusarium solani Phylosticta phaseolina were recorded from seeds of kidney bean (Gupta and Saharan,1973). Ascochyta infected bean seeds when sowed produced lesions on plants to the extent of 2 to 15 percents. (Hewett,1973). Ellis et al (1976) reported a correlation between seed mycoflora

and percentage germination of bean seed. Harrison (1978) discussed the role of seed-borne inoculum in the epidemiology of Botrytis fabae on field beans.

Ascochyta complex, Fusarium wilt and bacterial blight caused by Ascochyta spp., Fusarium oxysporum f. sp. pisi and Pseudomonas pisi on pea are responsible for heavy loss every year. In the U.S.A. average annual losses of the national production of green peas for the period 1951-1960 (Anon,1965) were estimated to 2 percent for Ascochyta complex, 2 percent for Fusarium wilt and 1 percent for bacterial blight. In Canada, Wallen (1965) found experimentally that A.pisi under certain conditions caused yield reduction to 11 percent, A. pinodella 25 percent and A. pinodes 45 percent. Teranishi and Namekata (1972) observed wilting in pea plant by Fusarium oxysporum f. sp. pisi.

Cicer arietinum is badly attacked by Ascochyta rabiei a causal agent of Ascochyta blight or leaf blight and some times Selerotinia sclerotiorum, stem and crown blight which are seed-borne in nature. In 1969 A.rabiei was found to cause severe dieback of foliage, and yields were so much reduced that it became a limiting factor in cultivation (Kaiser,1970). Halfon-Meiri (1970) observed a reduction in seed weight by 40 percent due to Ascochyta. Similarly losses ranging from 15 - 83 percent due to this disease has also been observed (Askerov, 1968; Morall and McKenzie,1974).

Amongst foliage and green vegetables the importance of cabbage and cauliflower can not be under estimated. The seeds of these vegetables are not free from fungi which are seed-borne such as Phoma lingam, dry rot, black leg and Xanthmmones compestris black rot and Alternaria brassicicola leaf spot. These are responsible not only for lowering the quality of seed but also decreasing the yield of seed. Giessmann and Daebler (1973) reported that Phoma lingam was a seed-borne pathogen on cabbage and was responsible for heavy losses. Heavy toll of various foliage vegetable crops due to seed-borne Alternaria has been reported from many parts of the world. (Holtzhausen,1978 and Wu et al., 1979). Singh et al.(1970) reported that Helminthosporium spiciferum and Curvularia lunata and leaf spot pathogens of egg plant are seed-borne and are responsible for heavy losses every year in India. Losses to the extent of 25% on okra due to Rhizoctonia bataticola and Choenophora cucurbitarum have been observed by Goel and Mehrotra (1973) Tai Luang Haun and Musa Bin Jamail (1975) and Geol and Mehrotra (1978). Fusarium oxysporum f. sp. spinaceae and Phoma vallerianellae are seed-borne (Bassi et al.,1978) and take heavy toll of the crop every year in France (Vegh et al.,1978).

It is evident from the above that seed-borne fungi are responsible of causing severe losses to vegetables in field by reducing the germination ability, resulting in damping off, seedling rot etc. Ultimately the crop is damaged and the yield is reduced. In India although considerable work has been done on seed mycoflora of vegetable seeds but the information with regard to vegetable seeds is still far from satisfactory. Therefore, in the present investigation an attempt will be made to determine seed mycolflora of selected vegetable seeds such as peas, cabbage, cauliflower etc. and to work out control measures to minimise the losses.

REVIEW OF LITERATURE

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Studies on seed pathology infact can be dated back to 1637 when Remnant reported that seeds carry disease causing agents. The pioneer work of Hellwig (1699) with Claviceps purpurea (Fr.) Tul. on rye, Micheli (1723) with Orbanche minor L. on beans and Tillet (1755) with Tilletia sp. on wheat established that disease causing agents are carried by seeds. Frank (1883) demonstrated that the bean anthracnose fungus Colletotrichum lindemuthianum was seed-borne in nature.

The first bacterial pathogen to be recorded as seed-borne was Xanthomonas phaseoli causing common bean blight (Orton, 1931).

Needham (1745) for the first time reported seed-borne nature of nematode Anguina tritici on wheat. This was followed by Kuhn (1857) who indicated seed-borne nature of Ditylenchus dipsaci.

Amongst the viral diseases lima bean mosaic was for first time suspected by Mcclintock (1916) as seed-borne. Subsequently several viral diseases have been reported seed-borne (Leach,1940; Carter,1966; Narayanaswamy and Jagannathen,1975).

Realising the importance of losses due to seed-borne diseases the first official seed testing station was established in Saxony in 1869 to determine the purity and germination capacity of seeds (Malone and Muskett,1964). However, before the establishment of seed testing station, the Switzerland government passed a law requiring the examination of seeds (Porter,1949). This was followed by realisation of losses due to seed-borne diseases in other countries. As a result of this more and more seed testing stations were established.

Muskett (1950) pointing out the significance of seed-borne diseases observed several fungi associated with seeds. Extensive studies on external seed-borne fungal flora of wheat were made by Christensen (1951) and of rice by Cherewick (1954). Kommendahl et al.(1955) while investigating the seed mycoflora observed Alternaria spp. and Colletotrichum spp. to be very frequent on seeds of flax. Schmidt (1955) reported 50 different species of fungi from certain agriculturally important crops. Detailed studies on seed-borne fungi on wheat (Russel 1956; De-Tempe,1964) pine seed (Gibson 1957) and small grains (Crosier,1961); guar, beans (Jain and Patil,1969), ground not (Joffe 1969; Gupta and Chohan,1971), Cucumbers, bottlegourd (Khandelwal and Prasad,1970), Cauliflower, bean and pea (Sekhon and Shivapuri,1971; Haware,1971) have been made. De-Tempe(1964a) pointed out that Fusarium sp. was seed-borne on cereals

of temperate countries. Phoma betae, Alternaria tenuis and Fusarium sp. were more frequent on sugarbeet seed (Gambogi,1964) and Alternaria dauci and Stemphylium redicinum on carrot (Hewett,1964); Ascochyta fabae on pea (Hewett, 1966) A.pisi, A.pinodella and Macrophomina phaseoli on pea (Bedlam,1985) A.rabei on chickpea (Halfon-Meiri 1970; Maden et al.,1975) was also found to be seed-borne. Seed-borne nature of the pathogen was also found to be for haloblight and anthracnose of bean. Watanabe (1972) established the seed-borne nature of large number of fungi on kidney bean, including some very serious. Aspergillus flavus was very frequently observed on the soybean (Dhingra et al.,1973); Rhizoctonia bataticola on okra (Goel and Mehrotra,1973). Scott and Wenham (1973) intercepted Alternaria radicina and A.dauci from the seeds of carrot imported to Newzealand. Tylkowska (1984) observed prevalence of large number of fungi including A.radicina and A.dauci on seven cultivars of carrot, while A.alternata was reported to be a serious pathogen of snap bean by Gomes and Dhingra (1983). In India Phoma betae, Cercospora bataticola and Verticillium spp. were intercepted in exotic lots of sugar beet seeds (Lambat et al.,1974) while Singh et al.(1974) isolated 17 genera of fungi. A comprehensive list of fungi associated with pea seed has been made by Bedi and Kapur (1974), while that of pepper and egg plant by Kar'kova et al. (1974); of cruciferous vegetable crops by Holtzhausen and

Knox-Davles (1974) and Toskova et al.(1975); of carrot by Mirkova (1976); of rape seed by Mills and Bollen (1976); of bottle-gourd by Dwivedi and Tandon (1976); of pulse crops by Suhag and Suryanarayan (1976); of bean by Lasca (1978), Kanapathipillai (1982) and Tylkowska (1984); of cruciferous crops by Tahvonen (1979) and Kanapathipillai and Hashim (1981); of soybean by Zad (1979) and Kumar et al.(1980); of cowpea by Kumari and Karan (1981); Zaidi and Prakash (1983) and Baross et al. (1985); of gram by D'Ercole and Sportelli (1982); of Raphanus sativus by Thakur et al.(1982) and of cucurbits by Komoto and Kimura (1982). Abdalla (1974) reported that although Aspergillus flavus and A.niger were frequently found in all the samples of seeds of ground nut but its frequency was very high in those having fractured shells. Pfleger and Herman (1975) observed Aspergillus flavus externally from pea seeds but it did not infect the embryo. Lorenzini et al.(1975) found Fusarium oxysporum f.sp. pisi isolated from seeds pathogenic and responsible for considerable damage. Khare (1975) pointed out that wilt in tentil, a seed-borne, could be controlled by antagonists, fungicides and irrigation routine management. It also adversely affected that germination of pea seeds (Lambhate and Bhide,1976). Peronospora manshurica was found to be seed-borne on soybean (Rosaca, 1976; Pathak et al., 1978) particularly in seeds

lots imported from the U.S.A. (Mukerwar et al.,1980). Another very important pathogen Glomerella sp. was also reported to be seed-borne on soybean by Roy (1982). Maude and Humpherson-Jone (1977,1980) observed that seeds of Brassica oleracea were heavily infected with A.brassicicola. Tripathi and Kaushik (1984) while studying the seed mycoflora of rape seed and mustard observed that intensity of infection of A.brassicae seed depended on the lesions on the fruits. Systemic seed transmission of another species of Alternaria i.e. A.sesami in sesame was reported by Dalbir Singh et al.(1983). Presley and Maude (1977a), Maude and Presley (1977) and Bochow (1980) pointed out that neck rot fungus Botrytis allii of onion was internally seed-borne and the infection persisted more than three years. Glushchenko (1980) observed that Peronospora destructor a seed-borne fungus caused epiphytotics of onion in the field. Dhingra (1978) concluded that Fusarium semitectum and Phomopsis spp. were internally seed-borne in bean seeds. Karwarsa and Mohinder (1982) were able to isolate 9 fungi and one bacterium (Xanthomonas cyamopsidis) from certified as well as local variety of cluster beans. Seed-borne nature of Rhizoctonia solani has been reported on cowpea (Diaz-Polanco et al.,1978) and beans (Wanzainum Wan Nik and Yap,1979). Kamal and Verma while isolating fungi from Pigeon pea seed observed that culture filtrates of some of

the fungi adversely affected the germination of seeds. Out of the three species of Aspergillus isolated from arhar seed by Shukla and Bhargava (1979), A.flavus proved to be pathogenic. Washings of Asparagus seeds were found to contain fungal bodies of Fusarium oxysporum f. sp. asparagi and F.moniliforme (Inglis,1979).

Damping-off in chillis caused by Colletotrichum capsici has also been reported to be transmitted through seeds (Grover and Bansal,1971). Colletotrichum sp. and Diaporthe phaseolorum var. sojae have been isolated from soybeans (Girard 1979) and Verticillium alboatrum from lucerne seed (Sheppard and Needham,1980). Christensen (1983) isolated V.alboatrum in 5 of 20 seed lots of alfa-alfa in Columbia. Srivastava and Gupta (1982) pointed out that seeds of Verbena hybrida collected from ten Indian locations were heavily infested with fungi and some of which were present in embryo and pericarp. Significance of seed-borne pathogens Macrophomina phaseoli, Cercospora cassiicicola and Alternaria sesami have been shown by Kushi and Khare (1979). Seed transmission of Macrophomina phaseolina on sesame was reported by Singh and Singh (1982). Out of three species of Fusarium isolated from tomato seeds, F.oxysporum var. herbarum proved to be pathogenic (Orlov et al.,1982) Alternaria alternata f.sp. lycopersici a causal agent of

tomato wilt epidemic was transmitted through seeds (Freshchini,1982). Seeds obtained from both healthy and blighted tomatoes of 3 field cultivars revealed the presence of Phytophthora infestans (Vartanian & Endo 1985). Inaba et al.(1983) while examining the seed washings to seed mycoflora observed the presence of Oospores of Peronospora farinosa a causal agent of spinach downy mildew in 6 of the eleven cultivars of commercial type studied.

Humpherson-Jones .(1943) observed that out of 30 commercial vegetable seed lots tested 13 were found infected with Alternaria brassicae and 15 with Leptosphaeria maculans. Fereira and Knox-Davles (1984) isolated Rhizopus sp. and Fusarium oxysporum from sweet melon seeds. Srivastava (1984) reported 35 fungi from 40 coriander seed lots. Of these about 17 fungi were pathogenic either to seed or at seedling stage.

The damage to seed by seed-borne pathogens is influenced by several factors. Christensen and Dorworth (1966) pointed out that moisture content of seed play an important role in the establishment of storage fungi in soybean seed. Christensen (1967) also noted an increase in infection by fungi on soybean at 25°C during storage. Similar results were obtained by Kudrina (1967) and Sanchez-Dominguez (1971) while working on seeds of vegetables. Soybean seed samples from different places in Illinois exhibited varied rate of

germination which was attributed to intensity of mycoflora (Tenne et al.,1974). A correlation of photoperiod on resistance of soybean seed with purple seed stain pathogen Cercospora kikuchi in Puerto Rico was noted by Hepperley (1984). Maude et al.(1985) reported that the leaf blight pathogen Alternaria dauci on carrot was favoured by warm, wet conditions and caused considerable damage by defoliation of plants.

Jensen (1967) reported R.carrotae as seed-borne and probably this was the first record of this disease on carrot. Several diseases of seed-borne nature on vegetables have been worked out by several workers e.g. Septoria spp. in celery (Hewett,1968), Botrytis spp. and Fusarium moniliforme var. subglutinosa (Lutynska, 1968 and Vannaci, 1981), Macrophomina phaseolina in cowpea and soybean (Sachston,1969 and Gangopadhyaya et al.,1970), Phoma spp. in pigeon pea (Lopezrosa,1969).

Antibiotics have also been found effective in minimizing the loss due to seed-borne diseases. Sowell (1965) by using streptomycin controlled Xanthomonas sp. on guar while Colletotrichum dematium var. truncata remained unaffected. Rauckyte (1965) could obtain inhibition of Ascochyta fabae on broad bean with nystatin and tetracyclin. Phytobacteriomycin seed treatment and trichothecin dusting on pea and chickpea reduced losses due Fusarium oxysporum and at the same time improved the germination (Kuzmina,1966). Biomycin, tetracyclin and terramycin reduced the growth of Pseudomonas lachrymans and Bacillus mesentericus in vitro (Avakyan and Gyusyan,1966). In addition to above 12 percent biomycin has been found effective against alternariosis of cabbage (Dorozhkin et al.,1972), griseofulvin and kusumin against verticillium wilt of cotton, griseofulvin and polyoxin against fusariosis of tomato, and griscofulvin against anthracnose of cucumber (Panteleev et al.,1973). Streptomycin against Pseudomonas pisi on pea (Taylor,1973); Penicillin or streptomycin against P.lachrymans (Kutova and Filipova,1977); takedamycin and kasugamycin against several fungi on cabbage, carrot, lettuce,radish and spinach, where as bacteria with agrimycin-100 (LO,1977); an antibiotic obtained from Streptomyces sp. 18 against Pythium debaryanum on tomato, lettuce and cabbage (Numić et al.,1979), fusamycin and sucamycin against root rot fungi on bean.

Simple treatment of seeds with water at room temperature and hot water treatment have been found to reduce the seed mycoflora (Tyner,1951). Maude (1966) observed that treatment of pea seeds with steam/air mixture at 65°C or 75°C killed the Ascochyta pisi and A.pinodes. Treatment of pea seeds infected with Ascochyta pinodes with UV radiation improved the germination (Peresypkin,1966). Hot water treatment has also been found effective against black leg agent Phoma lingam on cabbage (Williams,1967); against seed-borne cucumer mosaic virus (Sharma and Chohan,1971); damping-off and stage root rot of broad bean (Elarasi,1971); Phoma betae and other microbial contaminant on sugar beet (Herr, 1971; Lambat et al.,1974); Fruit rot Rhizoctonia spp. and Choenophora cucurbitarum on okra (Tai Luang Haun and Jamail, 1975); Peronospora parasitica downy mildew and black leg Leptosphaeria maculans on cabbage (Klinkovskaya,1976), against Macrophomina phaseolina and Fusarium equiseti on cowpea (Sinha and Khare,1977) Alternaria radicina on carrot (Utyugova and Levteeva,1977); Fusarium oxysporum f.sp. lagenaria on bottle gourd (Kuniyasu and Nakamura,1978) and Alternaria brassicae on mustard (Randhawa and Aulakh, 1984). In a modification, the hot water treatment has been combined with fungicide as well treatment which has considerably improved the germination (Wells and Merwarth,1973; Utyugova and Levteeva,1977).

Plant products have been reported very effective in minimising the seed mycoflora. Nene et al.(1968) tested antifungal activity of extracts of 88 plants against Helminthosporium turcicum of which 14 exhibited complete inhibition and nine partial inhibition in growth of the fungus. Khandelwal and Prasad (1968,70) used plant extract and fungicides for controlling the seed mycoflora of cucurbits. Kumar and Nene (1969) observed inhibition of Helminthosporium maydis and several other fungi by the extract of Cleome isocandra L. Staron et al.(1969) reported that triterpanic hiteroside (anagalloside) extracted from the stem and leaves of Anagallis arvensis var. pheenicea was inhibitory against Colletotrichum lindemuthianum and Pythium ultimum. Bambode and Shukla (1973) while testing the extracts of 36 plants against 6 pathogens found that pomegranate fruit rind, Plumbago zeylanica root bark adversely affected the growth of several fungi. Singh et al(1979) could free the gram seeds from wilt fungus Fusarium oxysporum f. sp. Ciceri and Sclerotinia sclerotiorum by treating with aquous extract of garlic seed.

Culture filtrates could also reduce the seed surface mycoflora. Dimovich et al (1971) succeeded in reducing the seed infection of pea from Fusarium oxysporum by treating the seed with culture filtrate of 2 species of Penicillium.

Vishunavat and Shukla (1981) reported that culture filtrate of Aspergillus fumigatus increased the germination of lentil and improved the seedling length. Fungi used as antagonists also reduced/controlled the seed borne pathogens in some crops. Akhtar (1969) pointed out that seed decay and damping off caused by Rhizoctonia solani on mustard could be controlled by the action of certain saprophytic soil-borne fungi. Roy and Abney (1979) observed a reduction in incidence of Diaporthe phaseolorum var sojae a seed-borne fungus when inoculated with Cercospora kikuchii. Windles and Kommendahl (1978) used Penicillium oxalicum to control seedling blight of pea. Herman et al (1980) observed reduction in the incidence of Pythium spp. or Rhizoctonia solani on radish and pea with Trichoderma hamatum. Wu and Lu (1984) pointed out that treatment of seeds with Gliocladium virens-19, Trichoderma harzianum-22, T.harzianum-50, Penicillium oxalicum-76 increased emergence and improved seedling health in crucifers and protected them against Alternaria brassicicola. Lifshitz et al (1985) obtained reduction in the incidence of Rhizoctonia pre-emergence damping off of radish by treating seeds with conidial suspension of T.harzianum.

Seed mycoflora can be controlled by several means i.e. physical and chemical means. Mechanical separation of fungal propagules and other debris has been practised for a long time (Anonymous,1966).

Hot water treatment of seeds has given a good control of seed mycoflora. The basic idea behind heat treatment is that dormant organs such as seeds, bulbs, tubers etc. used as propagative material can withstand higher temperature than those in which their respective pathogen can survive for a given period of time. The temperature of the hot water used and the duration of the treatment varies with different host pathogen combinations. Thus to control loose smut of wheat the seeds are recommended to be treated with hot water for eleven minutes at 58°C (Agrios, 1969). Whereas bulbs are treated at 43°C for 3 hours to get rid off Ditylenchus dipsaci (Agrios,1969). Low temperature treatment does not kill the pathogen but inhibit or retard the growth and activities thereby prevent the spread of existing infections and initiation of new ones (Agrios,1969).

Seed treatment with chemicals has been used for centuries for controlling plant pathogens. As early as 1660, salt brine was accidentally discovered to control the bunt of wheat. At the end of 18th century Abb'e Tessier in France tested many chemical compounds to improve wheat seeds,

without affecting agricultural practice (as quoted by Woolmen and Humphrey,1924). It was Prevost (1807) who demonstrated that copper sulphate could be used against bunt of wheat. It is this study which triggered research for finding out more and more chemicals to control seed-borne pathogens. Formaldehyde was later found to be a good and affective for seed treatment against various seed-borne fungi, particularly smuts (Geuter,1895; Bolley,1897). Copper carbonate was found satisfactory as dusting material for controlling wheat smut (Darnell - Smith 1910, 1915-1917). The modern era of fungicides and seed treatment started with the introduction of the organo-mercuric compounds. There has been tremendous amount of literature on this aspect which has been reviewed by Horsfall and Howard,1959.

It is difficult to summarise all what has been done on chemicals as seed treatment but in the following pages attempt has been made to tabulate some of the information available of chemical seed treatment against seed mycoflora of vegetables.

BENZIMIDAZOLE FUNGICIDES (SYSTEMIC)

Chemical name :- Methyl-N-(1-butylcarbamoyl)-2-benzimidazole carbamate

Trade name :- Benomyl/Benlate

Crop	Disease/pathogen	dosage	References
Pea	*Ascochyta disease*	42.2-56.6g/ 12.7 kg seed	Maude and Kyle (1970)
"	Powdery mildew	0.3 - 0.5%	Jhooty and Behar (1972)
"	*Ascochyta pisi*	-	Maude *et al* (1973)
"	*Macrosphaerella pinodes*	400 g/kg seed	Yoshii (1975)
"	*Fusarium oxysporum* f.sp. *pisi*	-	Lorenzini *et al* (1975)
"	Root rots and Ascochyta disease	2kg/ton seed	Kotova (1977)
"	Seed mycoflora	-	Batalova and Zino'ev (1978)
"	*Fusarium oxysporum* f.sp. *pisi*	-	Utikar *et al* (1978)
Cucumber	*Ascochyta melonis*, *Colletotrichum lagenerium*	0.2 - 0.3%	Oliker (1972)
Sweet melon	*Fusarium oxysporum*	-	Fereirra and Knox-Davles (1984)
Onion and garlic	*Sclerotium cepivorum*	10-40g/kg seed	Lafon and Bugaret (1970)
"	*S.cepivorum*	150g/kg seed	Enwistle and Muna-Singhe (1973)
"	*Botrytis allii*	1 g/kg	Maude and Presley (1977)

Contd..

Crop	Disease/pathogen	dosage	References
Bean	Rhizoctonia solani	0.1%	Segura and Diazpolanco (1975)
"	Fusarium solani f.sp. phaseoli	-	Russel and Musa (1977)
"	Macrophomina phaseolina	-	Petkar et al (1977)
"	Fusarium spp.	-	Shukla and Bhargava (1978)
Broad bean	R.solani and F.oxysporum	4g / kg seed	Eisa and Brakat (1978)
Faba bean	Ascochyta faba	0.2%	Bernier (1979)
French bean	Phaeoisariopsis griseola	-	Sindhan (1984)
Bean	Chocolate spot	-	Bainbridge et al (1985)
Chickpea	Ascochyta rabeiei	-	Kaiser et al (1973)
"	A. rabeiei	-	Reddy (1980)
"	Fusarium oxysporum f.sp. ciceri and F.solani	-	Mani and Sethi (1984)
Pea	Fusarium oxysporum f. sp. pisi	-	Utikar et al (1978)
"	Seed mycoflora	-	Batalova and Zinov'ev (1978)
"	F.oxysporum f. sp. pisi	-	Shukla et al (1979)
Gram	Sclerotium rolfsii, Rhizoctonia bataticola and F.oxysporum f.sp. ciceri	-	Verma and Vyas (1977)
Tomato	Seed mycoflora	-	Nedumaran and Vidyasekharan (1981)

Chemical name :- 2-(4'-thiazolyl)-benzimidazole

Trade name :- Thiabendazole

Crop	Disease/pathogen	dosage	References
Cabbage	Phoma lingam	1,1.2 and 5 a.i/ kg seed	Maude et al (1973)
Onion	Botrytis cinerea	-	Presley and Maude (1980)
Bean	Fusarium solani f. sp. phaseoli	-	Russel and Musa (1977)
Chickpea	Alternaria rabeiei	-	Kaiser et al (1973)

OXATHIIN FUNGICIDES (SYSTEMIC)

Chemical name :- 5,6-dihydro-2-methyl-1,4-Oxathiin-3-carboxinilide
Trade name :- Vitavax/Carboxin

Crop	Disease/pathogen	dosage	References
Cucubrit	*Rhizoctonia solani*	-	Jhooty and Grover (1971)
Onion	*Urocystis magica*	25 g/kg	Crete and Tartier (1973)
Chillies	*Colletotrichum capsici*	-	Narain and Panigrahi (1972)
Bean	*Macrophomina phaseolina*	-	Petkar *et al* (1977)
"	*Fusarium* spp.	-	Shukla and Bhargava (1978)
French bean	*Phaeoisariopsis griseola*	-	Sindhan (1984)
Carrot	*Alternaria dauci*, *Stemphylum radicinum* and *S.radicinum* var. *petroselina*	200g/100 kg seed	Nikova (1979)
Gram	*Sclerotium rolfsii*, *Rhizoctonia bataticola* and *F.oxysporum* f.sp. *ciceri*	-	Verma and Vyas (1977)

Chemical name :- 2,3-dihydro-5-carboxanilido-6-methyl-1, 4-oxathiin-4, 4-dioxide
Trade Name :- Plantavax/Oxycarboxin

Chillies	*Colletotrichum capsici*	-	Narain and Panigrahi (1972)
Bean	*Uromyces appendiculatus*	-	Okioga and Joffer (1972)

ORGANIC MERCURIAL FUNGICIDES

Chemical name :- Ethylmercury/Phenylmercury acetate etc.

Trade name :- Cerasan Red/Cereson dry etc.

Crop	Disease/Pathogen	dosage	References
Cabbage	*Pythium aphanidermatum*, *Rhizoctonia solani* and *R.batalicola*	-	Grewal and Singh (1965)
Sugarbeet	*P. aphanidermatum*	2.5g/kg seed	Sen *et al* (1974)
Okra	*Rhizoctonia bataticola*	0.3 %	Vir and Gaur (1970)
"	*R. bataticola*	0.3 %	Goel and Mehrotra (1973)
Bottlegourd	Germinability of seed	-	Sohi (1976)
Bean	Glomerella glycines	-	Ahn and Chung (1970)
"	Seed mycoflora	-	Kaul (1973)
"	Macrophomina phaseolina	-	Petkar *et al* (1977)
Faba bean	*Fusarium* spp.	-	Shukla and Bhargava (1978)

Crop	Disease/Pathogen	dosage	References
	Chemical name :- Phenylmercury acetata + Ethylmercury chloride		
	Trade name :- Agrosan - GN		
Bean	Seed mycoflora	-	Kaul (1973)
"	M. phaseolina	-	Petkar et al (1977)
Faba bean	Fusarium spp.	-	Shukla and Bhargava (1978)
French bean	Phaeoisariopsis griseola	-	Sindhan (1984)
	Chemical name :- Ethyl Mercury chloride		
	Trade name :- Granosan/ 2% cerasan		
Pea and broad bean	Seed mycoflora	2-4kg/ton seed	Volkova (1964)
Cucurbit	Bacillus mesentricus and F.oxysporum var. niveum	3g/kg seed	Avakyan (1966)
Beet	Black leg disease	4kg/ton seed	Pozhar (1971)

ORGANIC SULPHUR COMPOUNDS

Chemical name :- Tetramethyl thiuram disulphide or bis disulphide

Trade Name :- Thiram

Crop	Disease/Pathogen	dosage	References
Pea and Broad beans	Seed mycoflora	2kg/ton seed	Volkova (1964)
Pea	*Ascochyta pisi*, *Helminthosporum sativum* *Sclerotinia fructigena* & *Puccinia triticina*	-	Ksendzova (1974)
"	Post and pre-emergence mortality		Bedi & Kapur (1974)
"	Root rots and Ascochyta disease	50%	Kotova (1977)
"	Germination and emergence of plant	100-400g/kg seed	Rivera (1982)
"	*Pythium ultimum*	-	Lewis and Lumsden (1984)
Cabbage	11 seed-borne pathogens	-	Mande *et al* (1969)
Brassicas	*Pythium* spp., *Peronospora parasitica*	-	White *et al* (1984)
Cucurbits	*Bacillus mesentricus* and *Fusarium oxysporum* var. *niveum*.	2%,8-9g/kg seed	Avakyan (1966)
"	Germination of seeds	300-500g/kg	Alkamper (1966)
Beet	Black leg disease	6kg/ton seed	Pozhar (1971)
Onion	Collar rot	-	Boguslawski (1967)
"	*Botrytis* spp.	-	Lutynska (1968)

Crop	Disease/Pathogen	dosage	References
Onion	Urocystis cepula	-	Macias (1970)
"	Urocystis magica	25g/kg seed	Crete and Tartier (1973)
Chillies	Colletotrichum capsici	0.2%	Chakravarti and Anil Kumar (1975)
"	Some important diseases	-	Aleksic' and Aleksic'(1976)
"	Colletotrichum dematium	0.2%	Siddiqui et al (1977)
"	Pre-and post emergence losses	0.3%	Jharia et al (1978)
Okra	Rhizoctonia bataticola	-	Goel and Mehrotra (1973)
Watermelon & Melon	Improved germination	-	Sohi (1976)
Vegetables	Bacteria and fungi on seed	-	Kolev et al (1976)
Bean	Seed mycoflora	-	Kaul (1973)
"	R.solani and F.oxysporum	4g/kg seed	Eisa and Brakat (1978)
Faba bean	Ascochyta faba	0.2%	Bernier (1979)
Carrot	Alternaria radicina and A.dauci	-	Soteros (1979)
Brinjal	Helminthosporium sp.	-	Sarode and Kadam (1977)
Beet	Damping-off	-	V'rbanov et al (1984)
Onion	Microflora of seed	-	Kaul (1972)
Coriander	Mycoflora of seed	0.3%	Srivastava (1984)

Contd...

Crop	Disease/Pathogen	dosage	References
Gram	Seedling rot	-	Shrisat and Kale (1979)
	Chemical name :- Zinc ethylene bisdithiocarbamate		
	Trade name :- Zineb		
Onion	Collar rot	-	Boguslowski (1967)
Chillies	Some important disease	-	Aleksic' and Aleksic'(1976)
Carrot	Sclerotinia, Botrytis and Rhizopus sp.	0.1%	Tasca and Hulea (1973)
	Chemical name :- Zinc dimethyl dithiocarbamate		
	Trade name :- Ziram		
Chillies	Colletotrichum capsici	-	Narain and Panigrahi (1972)
Okra	Fusarium wilt	0.3%	Kapoor (1978)
French bean	Phaeoisariopsis griseola	-	Sindhan (1984)
	Chemical name :- Manganes ethylene bisdithiocarbamate		
	Trade name :- Maneb/Mancozeb/Dithane-22		
Cabbage	Soft rot and curd disease	-	Skowronski (1976)
Beet	Phoma betae and Pythium ultimum	80%	Dorpoux et al (1966)
Gram	Seedling death	-	Gurdip Singh and Bedi (1980)
	Chemical name :- Ferric dimethyl dithiocarbamate		
	Trade name :- Ferbam/Hexaferb		
Onion	Botrytis spp.	-	Lutynska (1968)
Bean	Fusarium spp.	-	Shukla and Bhargava (1978)

HETEROCYCLIC NITROGENOUS FUNGICIDES

Chemical name :- N-trichloromethyl thio-4-cyclohexene-1,2 dicarboxide

Trade name :- Captan/Orthocide

Crop	Disease/Pathogen	dosage	References
Garden pea	bacterial and fungal pathogens	-	Boyadzhiev (1972)
Pea	*Fusarium* sp.	-	Kirik and Steblyuk (1972)
"	Post and pre-emergence mortality	-	Bedi and Kapur (1974)
"	*Fusarium oxysporum* f.sp. *pisi*	0.2%	Gangopadhyay and Kapoor (1976)
"	Pre-emergence damping-off by *Pythium ultimum*	-	Ohh et al (1978)
"	*Ascochyta* sp.	-	Kuhne (1983)
Cabbage	*Pythium aphanidermatum*, *Rhizoctonia solani* and *R.bataticola*	-	Grewal and Singh (1965)
Chillies	*Colletotrichum capsici*	-	Chakravarti and Anil Kumar (1975)
"	Some important diseases	-	Aleksic' and Aleksic'(1976)
"	*Phytophthora capsici*	-	Aleksic' *et al* (1976)
"	improvement in germination capacity	-	Sohi (1976)
Bean	*Glomerella glycines*	-	Ahn and Chung (1970)
"	*Colletotrichum lindemuthianum*	200g/100kg seed	Petrov (1972)
"	*Macrophomina phaseolina*	-	Petkar *et al* (1977)

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Crop	Disease/Pathogen	dosage	References
Carrot	Seedling emergence	0.3% W/W	Perry and Hegarty (1971)
Sugarbeet	Phoma betae, F.oxysporum and Penicillium sp.	600/100kg seed	V'rbanov (1973)
"	Black leg disease	600g/100 kg seed	V'rbanov (1974)
Beet	Damping-off	-	V'rbanov (1984)
Chickpea	Ascochyta blight	0.1%	Bhatti et al (1983)
"	A. rabeiei	0.1%	Bhatti et al (1984)
	Chemical name :- Cis-N-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide		
	Trade name :- Captafol/difolatan		
Pea	Fusarium oxysporum f.sp. pisi	0.1%	Gangopadhyaya and Kapoor (1976)
Tomato	Seed mycoflora	-	Nedumaran and Vidyasekharan (198
Beet	Pythium ultimum	80%	Dorpoux et al (1966)
Chillies	Colletotrichum capsici	-	Chakravarti and Anilkumar (1975)
"	Phytophthora capsici	-	Aleksic' et al (1976)
"	Colletotrichum dematium	0.25%	Siddiqui et al (1977)
Okra	Rhizoctonia root rot	-	Chauhan et al (1979)

MISCELLANEOUS FUNGICIDES

Chemical name :- Pentachloronitrobenzene (PCNB)
Trade name :- Quintozene/Brassicol

Crop	Disease/Pathogen	dosage	References
Cucurbits	Rhizoctonia solani	-	Jhooty and Grover (1971)
Sweet melon	Rhizopus sp.	-	Fereirra and Knox-Davies (1984)
Sugar beet	Sclerotium rolfsii, Rhizoctonia solani	5g/kg seed	Sen et al (1974)
Beet	R.solani	-	Natti (1966)
Broad bean	R.solani and Fusarium oxysporum	4g/kg seed	Eisa and Brakat (1978)
Carrot	Sclerotinia, Botrytis and Rhizopus sp.	0.1%	Tasca and Hulea (1973)
Onion	Microflor of seed	-	Kaul (1972)
Okra	Rhizoctonia bataticola	-	Goel and Mehrotra (1973)
Gram	Seedling emergence	-	Shirsat and Kale (1979)

Chemical name :- 2,6-dichloro-4-nitroaniline
Trade name :- Bicloran/Botran

Onion	Sclerotium cepivorum	30g a.i./kg seed	Lafon and Bugaret (1968)
"	S.cepivorum	150-300g/kg seed	Enwistle and Muna Singhe (1973)

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

Seed samples of cauliflower and pea will be collected from the farmers field in and around Aligarh. Seeds will also be procured from National Seed Corporation and Indian Agriculture Research Institute, New Delhi. Seeds so obtained will be stored in air tight containers of P.V.C. Jars.. Initially the seeds will be subjected to visual examination and examination under the microscope after soaking in water for 12 hours, for the presence of contaminants (Conidia, pycnidia and mycelial fragments) on the surface.

Seeds will be transferred in water contained in 100 ml flask and shaken vigorously. The washing so obtained will be centrifuged to obtain the spore/fungal mass. The clear supernatant will be discarded and the residue will be examined microscopically for the presence of the fruiting body.

External seed mycoflora will be studied by using Blotter and Agar plate methods.

1. **Blotter Methods** :-

Moistened sterilized blotting papers will be placed in sterilized Petridishes of 9 cm. diameter. In each Petriplates equal number of seeds will be placed equidistantly

The petridishes will be incubated at 20 ± 2°C for 8 days with cycles of 12 hours light and 12 hours darkness. After 8 days of incubation fungi developing on the seeds will be isolated and identified.

2. Agar Plate Method:-

The seeds will be transferred in Petridishes of 9 cm diameter containing plain agar medium at equal distance. Incubation will be done as in the case of Blotter Method.

For internal seeds mycoflora both Blotter and Agar Plate Methods will be used. The seeds will be surface sterilized in 1:1000 mercuric chloride for 2-5 minutes followed by washings with sterilized water and will be transferred to Petriplates. Throughout the studies the plates will be incubated at 20±2°C. During the course of examination, the percentage germination of seeds and frequency and relative abundance of fungi will be determined. In these studies more attention will be paid to the mycoflora of structurally abnormal seeds. In internally seed-borne mycoflora, the site of infection in the seeds will be determined. Surface sterilized seeds with 1:1000 mercuric chloride will be placed in Petridishes containing plain agar and incubated at 20±2°C. The seeds showing mycelial infection will be boiled in KOH for 5 - 10 minutes till

they appear soft and translucent. The seeds will be given several washing with lactophenol and will be macerated and examined under the microscope.

In order to determine the pathogenicity of fungi appearing in these studies the seeds (100 in number) after having surface sterilized will be inoculated by placing them over the culture of potential pathogen grown on PDA and will be transferred to Petriplates. Equal number of inoculated seeds will also be kept to serve as control. Germination percentage and the development of symptoms if any will be observed during the course of these studies. In case fungi show some potential for pathogenesis the pathogenicity will also be determined at seedling stage. The seedlings will be inoculated by pouring suspension of the 5 gram fungus into the soil.

Effect of culture filtrates of pathogenic fungi on germinability of seeds will also be tested. The fungus will be grown in Richards medium contained in 250 ml. flask for 15 days. Surface sterilized seeds (100 in number) will be soaked in fungal culture filtrate for 24 hours. After soaking,seeds will be washed with sterilized distilled water and placed in sterilized Petriplates containing moist blotter paper. Appropriate number of seeds will be treated with sterilized distilled water to serve as control. Number of seeds germinated will be recorded as to calculate the percentage germination.

Attempts will be made to control the seed mycoflora with fungicides, nematicides,antibiotics and extract of certain plants known for antimicrobial activity. The seeds will be treated with fungicides, antibiotics and plant extract at different percentage of dilution and different time intervals and will be plated in sterilized petriplates. The plates will be incubated at 20±2°C for a week. The plates will be examined regularly for germination of the seed and the development of any fungi. Untreated seed will also be kept for control. Frequency and relative abundance of fungi will be calculated.

To test the effect of neem leaves on storage fungi, fresh neem leaves obtained will be oven dried at 60°C and will be cooled at room temperature. The leaves will be powdered and mixed with the seeds to be stored.

Through out the studies a lot of 400 seeds per sample will be taken unless stated otherwise. The data so obtained will be analysed statistically. The frequency and relative abundance of fungi will be calculated as under.

$$\text{Frequency} = \frac{\text{No. of seeds containing a particular fungus}}{\text{Total No. of seeds used}} \times 100$$

$$\text{Relative abundance} = \frac{\text{Total no. of colonies of a fungus}}{\text{Total no. of colonies of all the fungi}} \times 100$$

PLAN OF WORK

It is proposed to study the following aspects.

1. Survey of the seed-borne diseases of some important vegetable crops viz. cauliflower and peas.

2. Study of the external and internal mycoflora of cauliflower and peas.

3. Location of pathogens in the seed and their role in transmission of the diseases.

4. Pathogenicity studies of selected pathogenic fungi isolated from seeds of cauliflower and peas.

5. Effect of culture filtrate of important pathogenic fungi on germination of seeds of cauliflower and peas.

6. Effect of different parts and products of various plants known for antimicrobial activity on the seed mycoflora.

7. Effect of the above with fungicides on the seed-mycoflora.

8. Effect of dried leaves of neem (Azadirachta indica Juss.) on seed mycoflora.

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